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THE TUBERCULOCIDAL ACTION OF ARSENIC COMPOUNDS AND THEIR DISTRIBUTION IN THE TUBERCULOUS ORGANISM *

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS, XIV

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In the course of the study of problems relating to the chemotherapy of tuberculosis, we have investigated the value of arsenic compounds asserted by many clinical observers to be of definite value in the treatment of tuberculosis, and determined (1) the bactericidal action of arsenic on the tubercle bacillus, and (2) its distribution in the tuberculous organism.

HISTORICAL REVIEW

Arsenic has been used in the treatment of tuberculosis since ancient times. Dioscorides employed it internally and by inhalation. Antylus, who lived in the third century A. D., Marcellus Empyricus, and Galen all recommended it and described cures from the inhalation of powdered arsenic. It was also employed by the Chinese, and thence by the Hindus, who were acquainted not only with the oxid but also with the sulfur compound, realgar. A complete and interesting historical sketch of the use of arsenic in therapeutics, especially in tuberculosis, has been published by Kock.¹

In a recent study of the action of arsenic in tuberculosis Burow² found that potassium arsenite prevented the growth of tubercle bacilli in vitro. He further held that rabbit blood serum from an animal treated with guaiacol-arsenic (sodium and potassium guaiacolate each 1.5% with 0.01% potassium arsenite) prevented the growth of tubercle bacilli. This combination was given to tuberculous rabbits and guinea-pigs with, it is asserted, specific effect, altho the treated animals showed evidence of tuberculosis. The work of Burow cannot be said to have proved a specific action of the arsenic if one considers the variations which occur after inoculation of animals of the same species. The results of Burow have been contradicted by Nurnberger,³ who observed no specific action in tuberculosis, or on the tubercle bacilli in vitro.

Many clinical investigators have failed to demonstrate any specific effect of arsenic compounds in tuberculosis, among them being Knothe,⁴ Plicque,⁵ Grodecki⁶ (arsenical serum), Darthenay⁷ (arrhenal), Jacobi⁸ (arsenous acid

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¹ Nord. Med. Ark., 1902, 35, Afd. 2.

² München. med. Wechnschr., 1910, 57, p. 1792.

³ Ibid., 1911, 58, p. 2669.

⁴ Wien. klin. Wechnschr., 1911, 24, p. 562.

⁵ Jour. de méd. int., Paris, 1904, 8, p. 294.

⁶ Przegl. lek., Krakow, 1897, 36, p. 558.

⁷ Contribution à l'étude de l'action de l'arrhenal sur la nutrition des tuberculeux, 1912.

⁸ New York State Jour. Med., 1912, 12, p. 358.

with digitalis), Jolly,⁹ Schmey,¹⁰ Hoff¹¹ (arsenic cinnimate), and Vigenaud¹² (disodium methyl arsenite). Cybulski,¹³ using sodium arsenite, found no specific action in pulmonary tuberculosis. Bayeux¹⁴ found sodium cacodylate a valuable adjuvant in infancy and adolescence. M. Levrat¹⁵ and G. Levrat,¹⁶ after using sodium cacodylate in over 1,000 patients, concluded that it is not a panacea for tuberculosis, but that in certain cases it is an adjuvant of incontestable value. In fever the drug may be harmful. The findings of Kock,¹ Decombins,¹⁷ Jalaguier,¹⁸ Nicati,¹⁹ Anelli,²⁰ and Eysséric²¹ are in accord with these results.

Atoxyl, used with tuberculin by Mendel,²² and with ichthyol salicylate by Rohden,²³ was asserted by them to have a specific action on the tubercle bacillus, but they gave no experimental proof. They even concluded that salvarsan might be more specific. Renon and Delille²⁴ found no favorable effect in any forms of tuberculosis in human cases, or in tuberculous guinea-pigs.

That salvarsan has no specific action can be seen from the report of M. Bernay and A. Bernay,²⁵ whose results do not show any effects of this compound on tuberculosis. Lundie and Blaikie,²⁶ using sodium para-aminophenylarsonate (seomin), which contains 22.8% arsenic, found that it was not a cure for tuberculosis when given in deep injections.

To summarize the clinical reports in the literature, we may conclude that arsenic has not been demonstrated to have any specific action in tuberculosis, and that its value in this disease can be attributed only to its favorable effects on metabolism. Furthermore, in advanced tuberculosis it may even be harmful.

In order further to determine the effect of arsenic compounds on the tubercle bacillus, and their value in the treatment of tuberculosis, we have investigated the tuberculocidal action of sodium arsenite, sodium cacodylate, mercury cacodylate, atoxyl, arsacetin, and neosalvarsan, and have sought to determine whether or not these compounds enter the tissues of the tuberculous animal. A chemical substance to

⁹ Jour. de méd. de Paris, 1902, 14, p. 277.

¹⁰ Aertzl. Centr.-Anz., 1899, 11, p. 547.

¹¹ Ibid., 1899, 11, p. 530.

¹² Bull. gén. de therap., 1902, 143, p. 920.

¹³ München. med. Wchnschr., 1902, 49, p. 1393.

¹⁴ Cong. Internat. de la Tuberc., 1905, Paris, 1906, 2, p. 368.

¹⁵ Lyon méd., 1905, 104, p. 449.

¹⁶ Rev. de méd., 1911, 31, p. 493.

¹⁷ Le cacodylate de soude à doses massives et espacées dans le traitement de la tuberculose pulmonaire, 1914.

¹⁸ Le cacodylate de soude dans la tuberculose pulmonaire et quelques autres affections, 1901.

¹⁹ Le cacodylate de soude dans la tuberculose pulmonaire (Etude thérapeutique et pharmacodynamique), 1902.

²⁰ Riforma med., 1901, 17, p. 196.

²¹ Traitement de la tuberculose pulmonaire par le cacodylate de strychnine à hautes doses, 1902.

²² München. med. Wchnschr., 1909, 56, p. 13.

²³ Memoranda medica, 1903.

²⁴ Bull. gén. de therap., 1907, 154, p. 7.

²⁵ Jour. de méd. de Paris, 1912, 24, pp. 653, 975.

²⁶ Brit. Med. Jour., 1910, 1, p. 196.

be of value in tuberculosis must not only possess a specific germicidal or inhibitory action on the tubercle bacillus, but it must be able to permeate the avascular tubercle or be deposited there in sufficient concentration to exert its specific action. These problems have been discussed in the previous articles by Wells, Corper, and others.

ACTION OF ARSENIC COMPOUNDS ON TUBERCLE BACILLI IN VITRO

For determining the tuberculocidal action of arsenic compounds an emulsion of human tubercle bacilli was treated at 37 C. with various dilutions of each arsenic substance for a period of 24 hours. This length of time was adopted that we might ascertain from the beginning the value of these compounds as tuberculocidal agents; for a compound which is unable to cause death of the tubercle bacilli in 24 hours under favorable conditions could hardly be expected to be of value in such unfavorable conditions as obtain in the organism. If the arsenic compounds are unable to kill the tubercle bacilli in 24 hours, then injection of these treated bacilli will produce tuberculosis in the guinea-pig. If tuberculosis is not produced, then the compound has either killed the tubercle bacilli or inhibited their growth. In the latter case it is necessary to remove the chemical agent or neutralize its action before injection into animals.

In all the tests, results of which are given in Tables 1 to 6, the same technic was employed. To each tube containing 5 c.c. of solution were added 3 drops of a heavy suspension of human tubercle bacilli. These tubes were incubated at 37 C. for 24 hours. At the end of this time the bacilli were centrifugated and washed with salt solution and then injected subcutaneously into the inguinal region of normal guinea-pigs.

The Tuberculocidal Action of Sodium Arsenite.—We see from Table 1 that sodium arsenite in dilutions of from 0.1% to 0.0001% has no germicidal action on the tubercle bacillus in vitro in 24 hours at 37 C. All the animals showed evidence of generalized tuberculosis, and the differences were such as are expected in any series of inoculated animals. There is not even evidence of any inhibitory effect on the human tubercle bacillus.

The Tuberculocidal Action of Sodium Cacodylate.—Table 2 shows that sodium cacodylate in dilutions of from 2% to 0.002% has no germicidal action on human tubercle bacilli in vitro. In this series all the animals developed generalized tuberculosis, altho the animals injected with tubercle bacilli treated with the more concentrated solutions lived longer.

TABLE 1
THE TUBERCULOCIDAL ACTION OF SODIUM ARSENITE

Dilution of Sodium Arsenite	Duration of Life	Liver	Spleen	Lungs	Local Lymph Glands	Miscellaneous
0.1%	172 days..	Multiple foci of miliary tubercles	Enlarged; few miliary tubercles	Miliary tubercles; hemorrhagic	Large gland 2 cm. in diameter; local ulcer	Retroperitoneal glands caseous
0.01%	84 days..	Miliary tuberculosis	Numerous tubercles	Appear normal	Large tubercle 2 cm. in diameter	
0.001%	116 days..	Necrotic caseous areas	Five times normal size; necrotic caseous areas	Few miliary tubercles	Large local gland; local ulcer	Retroperitoneal and peribronchial glands enlarged
0.0001%	62 days..	Multiple tubercles and areas of necrosis	Greatly enlarged; necrotic caseous areas	Tubercles throughout; hemorrhagic	No enlarged local or retroperitoneal glands	Tubercular peritonitis

The kidneys were normal.

TABLE 2
THE TUBERCULOCIDAL ACTION OF SODIUM CACODYLATE

Dilution of Sodium Cacodylate	Duration of Life	Liver	Spleen	Lungs	Local Lymph Glands	Miscellaneous
2%	Killed after 152 days	Necrotic tuberculous areas	Enlarged miliary tubercles	Numerous tubercles	Caseous, greatly enlarged	Retroperitoneal and peribronchial glands enlarged
0.2%	152 days..	Numerous necrotic areas	About 6 times normal size; largely necrotic	Few small tubercles	Large ulcer; caseous glands	Peribronchial glands caseous; retroperitoneal glands enlarged
0.02%	137 days..	Miliary tubercles	About 10 times normal size; entirely necrotic	Small tubercles; hemorrhagic	Enlarged, caseous	Peribronchial and retroperitoneal glands enlarged
0.002%	56 days..	Several large necrotic areas	About 10 times normal size; entirely tuberculous	Numerous tubercles from 1 to 5 mm. in diameter	Large tubercle; caseous	Enlarged peribronchial glands

The kidneys were normal.

The Tuberculocidal Action of Mercury Cacodylate.—Table 3 indicates that mercury cacodylate in dilutions up to 0.001%, has a germicidal action on human tubercle bacilli. The death of the first animal, which received the injection of tubercle bacilli treated with 1% mercury cacodylate, was undoubtedly due to the toxic action of the mercury cacodylate, which had not been entirely removed in centrifugation. As the suspensions in this series had been centrifugated, and the upper 4 c.c. of cacodylate removed and replaced with salt solution, evidently 1 c.c. of the mercury cacodylate (1%) was toxic for the guinea-pig. The hemorrhages in the lungs, intestines, and at the site of injection were caused by the mercury cacodylate.

TABLE 3
THE TUBERCULOCIDAL ACTION OF MERCURY CACODYLATE

Dilution of Mercury Cacodylate	Duration of Life	Liver	Lungs	Kidneys	Local Lymph Glands	Miscellaneous
1%	3 days....	Hyperemia; centers of lobules distinct	Hemorrhagic, edematous	Pale, slightly swollen	Normal.....	Subcutaneous hemorrhages at site of injection; hemorrhages in large intestine
0.1%	Killed after 140 days	Normal.....	Normal.....	Normal.....	Slightly enlarged; no caseation	
0.01%	Killed after 140 days	Normal.....	Normal.....	Normal.....	Normal	
0.001%	Killed after 140 days	Normal.....	Normal.....	Normal.....	Normal	

The spleen was normal.

The relation of the toxic dose of this drug to the amount necessary to kill the tubercle bacillus remains to be determined. Like that of other mercury compounds, the action of mercury cacodylate in the animal's body would probably be less marked.

The fact that sodium cacodylate has no tuberculocidal action indicates that the mercury cacodylate owes its effect to the presence of the mercury in the molecule. DeWitt and Sherman²⁷ have found that mercuric chlorid in dilution of 0.001% has a germicidal action on the tubercle bacillus in 24 hours.

²⁷ Jour. Infect. Dis., 1914, 15, p. 245.

TABLE 4
THE TUBERCULOCIDAL ACTION OF ATOXYL

Dilution of Atoxyl	Duration of Life	Liver	Spleen	Lungs	Kidneys	Local Lymph Glands	Miscellaneous
1%	Killed after 168 days	Fatty changes; few tubercles	Adherent to surrounding tissues; enlarged, caseous	Numerous miliary tubercles	Few miliary tubercles	Enlarged, caseous	Peribronchial glands greatly enlarged
0.1%	104 days	Few miliary tubercles	Enlarged; necrotic tubercles	Miliary tubercles	Appear normal	Enlarged, caseous	
0.01%	Killed after 152 days	Caseous tuberculosis	Enlarged; miliary tubercles	Few miliary tubercles	Appear normal	Enlarged; no caseation	
0.001%	Killed after 168 days	Cirrhotic; fatty changes; minute tubercles	Enlarged; numerous tubercles	Multiple tubercles	Appear normal	Enlarged, caseous	Retroperitoneal glands enlarged, caseous; mammary glands caseous

TABLE 5
THE TUBERCULOCIDAL ACTION OF ARSACETIN

Dilution of Arsaceticin	Duration of Life	Liver	Spleen	Lungs	Kidneys	Local Lymph Glands	Miscellaneous
1%	Killed after 137 days	Miliary tubercles	Enlarged; caseous	Appear normal	Appear normal	Enlarged, caseous	
0.1%	143 days	Numerous tuberculous necroses	About 10 times normal size; many tubercles	Many large pale areas of tuberculosis in all lobes	Pale; no tubercles	Enlarged, slate-colored	Retroperitoneal and peribronchial glands enlarged
0.01%	115 days	Many necrotic areas	About 8 times normal size; mostly necrotic	Numerous large necrotic areas in lower lobes; consolidated	Appear normal	Enlarged, caseous	Peribronchial and retroperitoneal glands enlarged
0.001%	Killed after 137 days	Few minute whitish spots on surface	About 2 times normal size; few tubercles from 3 to 4 mm. in diameter	Numerous miliary tubercles	Appear normal	Enlarged, caseous	Pleural cavity contains bloody fluid

The Tuberculocidal Action of Atoxyl.—Atoxyl in dilutions of from 1% to 0.001% has no germicidal action on human tubercle bacilli in vitro. All the inoculated animals developed generalized tuberculosis. The results, given in Table 4, indicate that no conclusions can be drawn regarding the specific action of a drug on one organism from its

effects on another. The fact that atoxyl is specific for certain trypanosomes does not indicate that it will have any action on other species of micro-organisms.

The Tuberculocidal Action of Arsacetin.—Table 5 indicates that arsacetin in dilutions of from 1% to 0.001% has no germicidal action on human tubercle bacilli in vitro in 24 hours at 37 C. All the animals presented generalized tuberculous lesions.

The Tuberculocidal Action of Neosalvarsan.—Neosalvarsan, in dilutions of from 1% to 0.001%, has no germicidal action toward human tubercle bacilli in vitro in 24 hours at 37 C. All the animals presented generalized tuberculosis. The statements which occur in clinical literature suggesting a specific action of this drug in tuberculosis are therefore without foundation. One would hardly expect neosalvarsan to have a specific action toward the tubercle bacillus, an organism of a different group from that of *Spirochaeta pallida*, with different chemical composition and different biologic properties.

TABLE 6
THE TUBERCULOCIDAL ACTION OF NEOSALVARSAN

Dilution of Neosalvarsan	Duration of Life	Liver	Spleen	Lungs	Kidneys	Local Lymph Glands	Miscellaneous
1%	87 days	Numerous necrotic areas	Enlarged; miliary tubercles	Numerous tubercles	Appear normal	Enlarged; local ulcer	
0.1%	41 days	Several large necrotic areas	Eight times normal size; necrotic tubercles	Numerous tubercles from 1 to 4 mm. in diameter	Appear normal	Large, caseous	Peribronchial and retroperitoneal glands enlarged
0.001%	112 days	Many tubercles	Enlarged; necrotic	Appear normal	Appear normal	Enlarged, caseous	
0.001%	112 days	Many tuberculous necrotic areas	About 10 times normal size; entirely necrotic	Numerous miliary tubercles; pneumonic	Pale, fatty; no visible tubercles	Enlarged, caseous, pigmented	Peribronchial and retroperitoneal glands enlarged

ANALYTIC METHOD OF ASCERTAINING THE PRESENCE OF ARSENIC IN TISSUES OF INJECTED ANIMALS

Since in the following analyses we are dealing with small amounts of arsenic and are interested in determining the relative rather than the absolute amount present in the tissues, the following method, which is accurate for small amounts and does not require a great deal of time for performance, was used. It is a modification of the

Marsh-Berzelius method. The Sanger-Black modification of the Gutzeit method²⁸ was tried in control analyses on tissues containing known amounts of arsenic, but proved inadequate, being less accurate and less delicate than the following modified Marsh method, and being accompanied by numerous pitfalls when organic matter was present.

METHOD

The method to be described revealed an absolute agreement between mirrors directly marshed, with the use of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, and 30 micro-milligrams of arsenic and the same amounts of arsenic carried through the entire analytic process, in each case 10 grams of liver or 5 to 10 grams of arrowroot starch being employed. All reagents used in these analyses must give no appreciable arsenic mirror; that is, all of them together carried through the steps of the analysis must not give a mirror greater than 1 mmg. of arsenic. Those used in the following analyses gave only a faint trace—far less than 1 mmg.

Ten to fifteen grams of the tissue to be analyzed are weighed into a 4- to 5-inch porcelain evaporating dish or casserole, with a watch glass cover, and 10 c.c. of 1.84 sulfuric acid added; this suffices to preserve the tissue until the organic matter can be destroyed. When the worker is ready to destroy the organic matter, the evaporating dish is put on an air bath (having a thermometer at one side) so that only a small edge of the dish projects above the bath. The bath must be in a well-ventilated hood or else proper suction apparatus must be arranged to remove the nitric acid fumes. Then, 20 to 30 c.c. of 1.42 nitric acid are added cautiously and gentle heating begun, for a rapid violent evolution of NO_2 and frothing may otherwise occur which might occasion loss of some of the material. After the first violent frothing and evolution of NO_2 have subsided, the heating may be resumed and the temperature of the air bath brought to about 160 to 180 C. With the recurrence of charring, repeated additions of from 3 to 5 c.c. nitric acid are immediately made (charring should not continue too long without the addition of nitric acid, as there must be no formation of SO_2) until the liquid no longer turns brown but remains pale yellow—due to ferric chlorid—or colorless; a few more additions of from 2 to 3 c.c. of nitric acid are then made to insure the destruction of all organic matter, and heating increased until the appearance of white sulfuric acid fumes. Since in different air baths the temperature at which this occurs varies, only an approximate temperature can be given—about 180 to 200 C.—but the best index is the first appearance of heavy white fumes. If with the occurrence of these fumes there is no more browning of the solution, it is cooled and from 10 to 15 c.c. of distilled water added, and heat applied to boil off the water until the reappearance of white fumes. Sometimes browning again occurs at this stage and then it is necessary to make a few more additions of nitric acid. Three additions of from 10 to 15 c.c. of distilled water and heating until white fumes appear, generally suffices to free the solution from nitric acid, but to make sure of this a few drops of the liquid are added to a diphenylamin sulfate-sulfuric acid test solution²⁹ (1 part of nitrogen in 20,000,000 parts of water gives a violet-blue color with a drop of diphenylamin sulfate in sulfuric acid followed by 2 c.c. concentrated

²⁸ Jour. Sociol. Chem. Industry, 1907, 26, p. 1115.

²⁹ Watts: Dictionary of Chemistry, Vol. 3, p. 558.

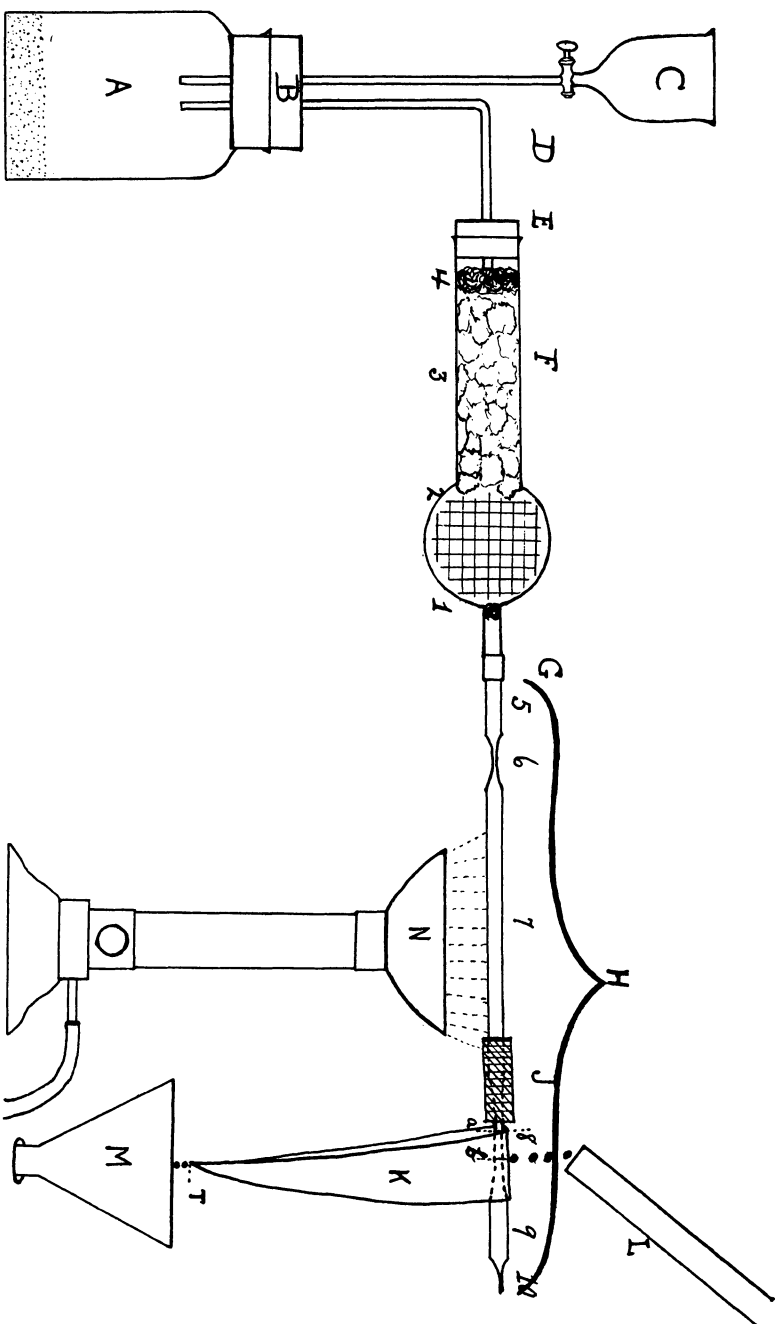


Fig. 1. The apparatus used in "marshing."

A = wide-mouthed bottle, capacity 100 to 200 c.c.
 B = glass or rubber stopper.
 C = dropping funnel with stop-cock.
 D = delivery tube.
 E = rubber stopper.
 F = calcium chlorid tube containing, at 1, a glass wool plug; at 2, a filter paper or absorbent cotton saturated with lead acetate; at 3, neutral granulated calcium chlorid; and at 4, another glass plug.

G = rubber tubing connecting F to H.
 H = glass depositing tube.
 L = cold water conduit.
 K = nickel gauze.
 M = filter paper.
 N = collecting funnel.

sulfuric acid and stirring) on a porcelain plate. The sulfuric acid solution free from all nitric acid and containing the arsenic is ready for "marshing."

The method used for "marshing" and preservation of the mirrors is a modification of the method used by Thomson.³⁰ The apparatus used consists of a wide-mouthed bottle (A) with a capacity of from 100 to 200 c.c., dependent on the amount of solution to be marshed, with a glass stopper (B)—vaselined—or a large rubber stopper (it may be a boiled-out rubber stopper) with double perforations containing a small dropping funnel (C) with a stop-cock, and an L-shaped delivery tube (D). The delivery tube (D) is inserted into a rubber stopper (E) which fits a small calcium chlorid tube (F) containing at 1, a glass wool plug; at 2, filter paper or absorbent cotton saturated with normal lead acetate of 1% solution and dry to remove hydrogen sulfid (this filter paper or cotton must be tested for lead acetate; when placed over the mouth of an ammonium sulfid bottle, it must turn black); at 3, neutral granulated calcium chlorid for drying the gas; and at 4, another glass wool plug. The calcium chlorid tube (F) is connected at its narrow end by means of a snugly fitting piece of rubber tubing (G) with the depositing tube (H)—a piece of hard glass tubing, medium-walled, from 6 to 7 mm. in external diameter, which will not collapse when heated to redness in the Bunsen flame. By means of a blast lamp the tube (H) is drawn down at 6 and 8—the constriction at 6 being made last, after measuring the internal dimension at 8 as directed hereafter—and drawn out to a tip at 10. The length of (H) is approximately 2 cm. at 5, 2 cm. at 6, 10½ cm. at 7, and 4½ cm. at 8. At 8 the tube is drawn down to about 3.0 to 2.9 mm. in external diameter and then measured internally by noting and marking with glass pencil the points at which two nails, 2.7 and 2.2 mm. in diameter measured by calipers, or wires are obstructed. The point of obstruction of the larger nail (a) is the site at which the deposition of the mirror is begun and is nearer the generator, while the point (b) at which the smaller nail is obstructed merely gives a gauge as to the range within which we can deposit the mirror so that there will not be too great a difference in the internal diameters in which the deposit occurs. The distance between the two points of obstruction should be about 1 cm. J is a small piece of nickel gauze (iron or copper gauze will serve tho somewhat unsatisfactory because easily melted or corroded) about 2 to 3 cm. long and wide enough to fit around the depositing tube (H) a little more than once. It is adjusted so that it will be about 2 mm. from the deposition line (a). K is a small piece of thin filter paper, lens paper, or tissue paper, about 2 to 3 cm. wide, cut so that folded it will hang over the tube (H) with its near edge on the line (a) and with two points meeting at (T), so that the cold water (10 to 20 C.) run onto it from above by means of the tube (L) connected to a cold water reservoir for the purpose of cooling the depositing tube (H) at 8 (the cooling is necessary to obtain a single and complete mirror), will run into the collecting funnel (M), which passes through a hole in the table and then into a large refuse bottle or drain pipe.

When the worker is ready for "marshing," about 20 grams of platinized granulated zinc (Baker and Adamson's platinized granulated zinc, Serial 2772, is satisfactory, sensitive and free from arsenic) are placed in the bottom of the generator (A), and about 10 to 15 c.c. of concentrated 1.84 stock sulfuric acid diluted with from 10 to 15 c.c. distilled water are run onto it through the funnel (C). This causes a violent evolution of hydrogen, which sweeps

³⁰ Chemical News, 1902, 86, p. 179; 1903, 88, p. 228; 1906, 44, p. 156.

all the air from the apparatus. In the meantime the solution to be "marshed" is placed in a small Erlenmeyer flask and brought to boil, removed from the flame, and the ferric iron reduced by the addition of a few crystals of fresh, chemically pure stannous chlorid (a rather large quantity will not inhibit the evolution of arsin, but a few crystals generally suffice) and shaken. Complete reduction occurs in about 5 minutes and is tested by adding a few drops of the solution to a solution of potassium sulfocyanate on a white porcelain plate. If reduction is not complete, the solution is warmed again, but not boiled, and more stannous chlorid added. When reduction is complete, the flask is stoppered and allowed to cool. The Marsh apparatus, free from air (tested by collecting the gas at 10 by upward displacement in a small test tube, and igniting) is tested for leaks by passing a small flame along its entire length, ignition occurring if leaks are present. The deposition tube is now freed from moisture by gently warming with a flame starting at 5 and passing to 10. The wing top Bunsen flame (N) is now lighted—blue flame—and placed so that it will heat the tube (H) red hot for about 7 cm. along 7, the flame touching the nickel gauze (J). Now the paper (K) is put into place and a small but fairly rapid stream of cold tap water is allowed to run over it. The evolution of hydrogen is gauged by the size of the hydrogen flame at 10, which must be about 1 to 2 cm. in length during the entire "marshing." When the violent evolution occasioned has subsided and the apparatus is ready, the unknown solution is poured into the dropping funnel (C), a few cubic centimeters at a time, and allowed to run into the generator. The evolution of hydrogen is regulated by adding a few cubic centimeters of distilled water to hasten, and stock sulfuric acid solution (equal parts of concentrated 1.84 sulfuric acid and distilled water) to retard it. (This was more satisfactory than the routine method of using certain amounts of three different concentrations of sulfuric acid.) The entire unknown solution should be run into the Marsh apparatus in about 10 to 20 minutes and "marshing" continued for 35 to 40 minutes after the last part of the unknown solution has been added. Tests have shown that practically all the arsin has passed quantitatively over by this time provided the generator A is not too large and the evolution of hydrogen is kept up. The flame (N) is then turned out and the tube (H) allowed to cool (keeping up the evolution of hydrogen). By means of a small blast flame the tube (H) is then first sealed at 10, the stop-cock of funnel (C) rapidly opened, and the tube finally sealed off at 6. The arsenic mirror thus sealed in hydrogen may be kept in the dark for long periods of time without deterioration. In the following analyses fractions of the unknown solution were taken which would give an arsenic mirror reading between 2 and 10 micromilligrams. The standard arsenic solution for making the control mirrors was prepared by dissolving 1.33 grams (or fraction thereof) of chemically pure arsenic trioxid in about 100 c.c. of boiling distilled water with the aid of sodium hydroxid or sodium carbonate added drop by drop until the crystals of arsenic trioxid have completely gone into solution, and diluting to one liter with cold, freshly boiled, distilled water. One cubic centimeter of this solution diluted to 100 c.c. gives the standard, one cubic centimeter of which equals 10 micromilligrams of arsenic. This diluted standard does not keep well, so that it must be freshly prepared at frequent intervals from the stronger solution.³¹

³¹ Clark and Woodman: Circular 99, U. S. Department of Agriculture, Bureau of Chemistry.

THE DISTRIBUTION OF ARSENIC IN THE TISSUES OF TUBERCULOUS
ANIMALS GIVEN VARIOUS ARSENIC PREPARATIONS

EXPERIMENT 1.—A rabbit (2800 gm.) was inoculated with human tubercle bacilli in the right eye and 143 days later, when the entire bulb of the right eye was involved with tuberculosis, was given intravenously 6 mg. of arsenic in the form of sodium arsenite in 6 c.c. of water; 2 days later another 6 mg. arsenic were given, and 1 day later another 6 mg. of arsenic. Eight hours after the last injection the animal was bled to death and the organs were analyzed. The normal left eye contained 1 mmg. arsenic in 3 gm.; tuberculous right eye 2 mmg. in 2 gm.; blood 3 mmg. in 20 gm.; spleen 3 mmg. in 2 gm.; right kidney 10 mmg. in 7 gm.; left kidney 21 mmg. in 8 gm.; lungs 6 mmg. in 9 gm.; and the liver 60 mmg. in 12 gm.

EXPERIMENT 2.—A guinea-pig was inoculated in the left groin with 0.05 mg. human tubercle bacilli and 58 days later was given subcutaneously 0.5 c.c. of a 2% solution of sodium cacodylate in physiologic salt solution, and on succeeding days 0.75 c.c., 1.0 c.c., 1.25 c.c., and 2.0 c.c., respectively. The animal was killed by bleeding 8 hours after the last injection and the tissues were analyzed. The peribronchial glands contained 21.7 mmg. of arsenic in 3.0 gm.; spleen 15.8 mmg. in 2.5 gm.; testes 18.8 mmg. in 3.0 gm.; blood 87.5 mmg. in 17.5 gm.; lungs 17.8 mmg. in 5.0 gm.; kidneys 21.2 mmg. in 4.0 gm.; and liver 32.0 mmg. in 8.0 gm. The inguinal glands were large and caseous; the peribronchial gland was hard and enlarged. The spleen was enlarged and full of numerous small necrotic areas; the liver pale and necrotic; the lungs, kidneys, and testes normal.

EXPERIMENT 3.—A guinea-pig was inoculated in the left groin with 0.05 mg. human tubercle bacilli and 58 days later was given subcutaneously 1 c.c. of 1% atoxyl in physiologic salt solution, the second day 1 c.c., the third day 2.0 c.c., the fourth day 1.5 c.c. (animal sick and becoming emaciated), and the fifth day 1.0 c.c. Eight hours after the last injection the animal was bled to death and the tissues were analyzed. The local lymph glands (caseous) contained 50.0 mmg. arsenic in 3.0 gm.; retroperitoneal glands 24.2 mmg. in 2.5 gm.; peribronchial glands 15.6 mmg. in 1.5 gm.; blood 94.0 mmg. in 6.0 gm.; spleen 31.3 mmg. in 2.5 gm.; testes 14.7 mmg. in 3.0 gm.; and the liver 68.9 mmg. in 11.0 gm. Local, retroperitoneal, and peribronchial glands were enlarged. The spleen was full of miliary tubercles. There were many small necrotic areas in the liver. Peritoneal hyperemia was noted. The animal was emaciated.

EXPERIMENT 4.—A guinea-pig was inoculated in the left groin with 0.05 mg. human tubercle bacilli and 30 days later was given subcutaneously 4.5 c.c. of 1% arsacetin solution and the next day 8.0 c.c. Two hours after the last injection the animal was bled to death and the tissues were analyzed for arsenic. The local inguinal glands contained 60.0 mmg. of arsenic in 2 gm.; peribronchial glands 60 mmg. in 2 gm.; blood 750 mmg. in 17.0 gm.; lungs 300 mmg. in 5 gm.; spleen 60 mmg. in 3 gm.; kidneys 750 mmg. in 6 gm.; and the liver 225 mmg. in 13 gm. The local retroperitoneal, and peribronchial lymph glands were enlarged. There were small necrotic areas in the liver; the spleen was full of necrotic areas; the lungs also showed a few such areas. The rest of the organs appeared normal.

EXPERIMENT 5.—A guinea-pig was inoculated in the left groin with 0.05 mg. human tubercle bacilli and 30 days later was given subcutaneously 4.5 c.c. of 1% neosalvarsan and the next day another 4.5 c.c. One and one-half hours

after the last injection the animal was bled to death and the tissues were analyzed for arsenic. The local caseous inguinal glands contained 12 mmg. of arsenic in 3 gm.; retroperitoneal glands 5 mmg. in 1 gm.; spleen 12 mmg. in 2 gm.; blood 50 mmg. in 19 gm.; lungs 50 mmg. in 4 gm.; kidneys 50 mmg. in 5 gm.; and the liver 50 mmg. in 10 gm. The local and retroperitoneal glands were enlarged; peribronchial glands only slightly so. The liver and the spleen were full of small necrotic areas, and the lungs showed a few small necrotic areas. The rest of the organs appeared normal.

As a result of these experiments it is noted that:

Arsenic in simple crystalline salt form, as sodium arsenite, sodium cacodylate, atoxyl, arsacetin, and neosalvarsan, administered to tuberculous animals parenterally, is found in the liver, lungs, kidneys, blood, spleen, and tuberculous tissues (lymph glands of guinea-pigs and eye of rabbit), the concentrations in the various tissues not greatly differing. No evidence of accumulation in the tuberculous tissues was obtained.

Incidentally, since tin forms salts like arsenic, in which the tin is in the negative radical, the following experiments were performed to test whether sodium stannate was germicidal toward the tubercle bacillus.

A uniform suspension of human tubercle bacilli was added in equal amounts (5 drops) to 5 c.c. each of the following concentrations of sodium stannate: 0.001, 0.01, 0.1, and 1.0%, all in duplicate. At the same time 7 controls were made with 5 c.c. distilled water, and placed in the incubator at 37 C. for 48 hours (shaken at frequent intervals). At the end of this time the suspensions were injected subcutaneously into the left groin of normal guinea-pigs. It suffices to state that all the guinea-pigs receiving the stannate-treated bacilli developed a marked tuberculosis which did not differ materially from that of the controls. Sodium stannate, even in concentrations as high as 1.0% for 48 hours at 37 C., is therefore non-germicidal toward the human tubercle bacillus; no evidence even of attenuation was observed.

SUMMARY AND CONCLUSIONS

Sodium arsenite in dilution of from 0.1% to 0.0001% and sodium cacodylate in dilution of from 2% to 0.002% have no germicidal action on human tubercle bacilli in 24 hours at 37 C.

Mercury cacodylate in dilutions of from 1% to 0.001% has a germicidal action on human tubercle bacilli in 24 hours at 37 C. This action is in all probability due to the mercury and not to the cacodylate radical.

Atoxyl, arsacetin, and neosalvarsan in dilutions of from 1% to 0.001% have no germicidal action on human tubercle bacilli in 24 hours at 37 C.

These compounds, representing the commonly used inorganic and organic preparations of arsenic, cannot be said to have any specific action on human tubercle bacilli, and if of value in the treatment of tuberculosis, are so only because of their favorable influence on metabolism. That tissue compounds would produce combinations with arsenic in the animal body which might be tuberculocidal is very unlikely, for a review of the clinical literature presents no evidence of any specific action of arsenic compounds in tuberculosis.

Arsenic in simple crystalline salt form—sodium arsenite, sodium cacodylate, atoxyl, arsacetin, and neosalvarsan—administered to tuberculous animals parenterally is found in the liver, lungs, kidneys, blood, spleen, and tuberculous tissues (lymph glands of guinea-pigs and eye of rabbit), the concentrations in all these tissues not greatly differing. No evidence of accumulation in the tuberculous tissues was obtained.

Sodium stannate, even in concentration as high as 1% for 48 hours at 37 C., is not germicidal toward human tubercle bacilli.